

IN THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS

1. (Currently Amended) A method for constructing a ~~functional~~ mammalian ~~organ~~ tissue or a fragment thereof in vitro, comprising:

(a) _____ culturing and propagating embryonic epithelial-derived explants, tissue or cells comprising:

(i) _____ isolating the tissues or cells and growing them in culture,

(ii) _____ permitting the ~~culture~~ tissue or cells to form multiple branches,

(iii) _____ dissecting out individual branch tips,

(iv) _____ reculturing the individual branch tips in the presence of medium, serum, at least one growth factor mix, and conditioned medium on an extracellular matrix (ECM) gel ~~and nutrient-rich medium~~ for several generations to generate branch tip buds;

(b) _____ ~~simultaneously~~ culturing and propagating isolated embryonic or fetal metanephric mesenchyme comprising:

(i) _____ dissecting out embryonic or fetal metanephric mesenchyme at the time of induction,

(ii) _____ culturing the embryonic or fetal metanephric mesenchymal tissue in the presence of medium, serum, at least one growth factor mix, and conditioned medium ~~and nutrient-rich medium~~,

(iii) partitioning the mesenchyme into multiple pieces and
~~growing~~ culturing each piece separately, and

(iv) inducing vasculogenesis by subjecting ~~grown~~ cultured
mesenchyme to substrate deprivation or addition ~~or~~ of soluble factors;

(c) recombining each vascularized mesenchyme with each cultured
branch tip bud in a matrix in which in vitro angiogenesis has begun; and

~~growing~~ culturing the combined tissue under conditions to ensure
continued vasculogenesis to obtain a vascularized mammalian tissue,

wherein the at least one growth factor comprises glial cell line-derived
neurotrophic factor (GDNF).

2. (Withdrawn) A method for in vitro culturing and propagating ureteric bud
tissue, comprising:

isolating embryonic kidney rudiments by dissection,
isolating ureteric bud tissue fragments from mesenchyme by
incubating said kidney rudiments with a proteolytic enzyme in the presence of
DNAse and/or by mechanical separation;
suspending said isolated ureteric bud fragments in a gel matrix;
placing the gel/fragment composition on porous polycarbonate membrane
inserts in wells of tissue culture plates;
adding growth factors to the culture wells;
maintaining the gel composition at the interface of air and medium until said
fragments form multiple tubular branches inside the gel matrix;
dissecting out distal individual branch tips formed during culture; and
reculturing said branched tips in the presence of serum, growth factor mix, cell
conditioned medium and nutrient-rich medium for several generations.

3. (Withdrawn) The method according to claim 2, wherein the mechanical
separation is accomplished by manual dissection.

4. (Withdrawn) The method according to claim 2, wherein the mechanical separation is accomplished by laser separation and capture.
5. (Currently Amended) The method according to claim 1, wherein the at least one growth factor mix comprises a glial cell line-derived neurotrophic factor or functional equivalent thereof and at least one other growth factor selected from the group consisting of EGF, HGF, IFG, and FGF-2.
6. (Previously presented) The method according to claim 1, wherein the added conditioned medium comprise a growth promoting constituent or inducer of differentiation or morphogenesis.
7. (Currently Amended) The method according to claim 1, wherein the matrix comprise a mixture of type I collagen and Matrigen a basement membrane preparation, or an equivalent matrix.
8. (Withdrawn) A method for in vitro culturing and propagation of metanephric mesenchyme, comprising:
 - dissecting out fetal kidney mesenchyme tissue [at the time of induction];
 - culturing said mesenchymal tissue in the presence of serum, growth factor mix, mesenchymal and/or bud cell conditioned medium and nutrient-rich medium;
 - partitioning the cultured mesenchyme into multiple pieces and growing each piece separately in culture; and
 - subjecting grown mesenchyme to substrate deprivation or addition of vasculogenic growth factor in order to induce vasculogenesis.
9. (Withdrawn) A method for in vitro engineering and constructing a mammalian kidney, comprising:
 - culturing and propagating a ureteric bud by
 - isolating the ureteric bud in culture,
 - permitting the culture to form multiple branches,

dissecting out the individual branch tips,
reculturing in the presence of serum, growth factor mix,
mesenchymal and/or bud cell conditioned medium and nutrient-rich medium
for several generations;

culturing and propagating isolated embryonic or fetal metanephric
mesenchyme by

dissecting out fetal mesenchyme at the time of induction,
culturing mesenchymal tissue in the presence of serum, growth factor
mix,

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medium and nutrient-rich medium,

partitioning the mesenchyme and growing each piece separately, and
inducing vasculogenesis by subjecting grown mesenchyme to
substrate

deprivation or addition of vasculogenic growth factors;
recombining each vascularized mesenchyme piece with each cultured
bud in a matrix in which in vitro angiogenesis has begun; and
growing in richest medium conditions to ensure continued
vasculogenesis.

10. (Currently Amended) The method according to claim 1, wherein the
~~tissues are~~ the vascularized mammalian tissue is implanted into a recipient without
prior induction of vasculogenesis.

11. (Withdrawn) A function mammalian kidney constructed in vitro from isolated
embryonic or fetal kidney tissue or cells that are cultured in rich medium having
present a mixture of growth factors and inducer substances, comprising:

an isolated ureteric bud propagated in culture to produce a functioning
nephron;

metanephric mesenchyme propagated from cultured embryonic
mesenchymal tissue fragments or cells; and

recombination of propagated ureteric bud and metanephric mesenchyme wherein said recombination in culture results in a functioning kidney or a functionally equivalent fragment thereof.

12. (Currently Amended) The method of claim 1, wherein the vascularized mammalian tissue is mammalian kidney tissue.